# THE FIBRILLAR SYSTEMS OF CILIATES AS REVEALED BY THE ELECTRON MICROSCOPE. I. PARAMECIUM [1]

CHARLES B. METZ, DOROTHY R. PITELKA AND JANE A. WESTFALL

*Dept. of Zoology, University of California, Berkeley 4, California, and Dept. of Zoology, University of North Carolina, Chapel Hill, N. C.*

For many years the fibrillar systems and associated organelles of protozoa have attracted the attention of cytologists and protozoologists. Initially this interest appears to have been directed by morphological and functional considerations. More recently and with the appreciation of the self-duplicating nature of these systems, their causal relation to morphogenesis, regeneration and the polarity of the organism has commanded first attention (Tartar, 1941; Fauré-Fremiet, 1948; Lwoff, 1950; Weisz, 1951). Unfortunately, these studies have been limited by the fact that the structures in question have dimensions which border on the resolving power of the light microscope. Thus, with the exception of cilia, flagella and exploded trichocysts (Jakus and Hall, 1946; Pitelka, 1949; Finley, 1951, and others) the detailed anatomy of the fibrillar apparatus and associated organelles has remained obscure. For an adequate understanding of the functional and morphogenetic properties of these systems a detailed picture of their fine anatomy would appear to be essential. Presumably such a picture could be obtained by an electron microscope examination of these systems in serial sections (Sedar, 1952) or preferably after appropriate dissection of the material. In the present study "sonic dissection" was found to be a simple and effective method of preparing fragments of the ciliate protozoan surface, together with the subjacent fibrillar apparatus, for electron microscope examination. The present effort, the first in a series of morphological studies employing this technique, should now provide a new point of departure for the investigation of the physiology of locomotion, coordination and developmental mechanics in ciliate protozoa.

The primary fibrillar apparatus of ciliates consists of a number of parallel structural units, the kineties (terminology of Lwoff, 1950). Each of the several kineties in turn is composed of: 1) a longitudinal row of cilia; 2) a longitudinal row of small granules, one granule at the base of each cilium (the ciliary basal bodies or kinetosomes); and 3) a longitudinal ectoplasmic fiber which connects all the basal granules in the row (the silver line or neuromotor fiber, neuroneme, or kinetodesma). As revealed by the light microscope, this unit of organization is an anatomically continuous structure. In the more complex ciliates this fibrillar pattern is rendered more involved by modifications in the arrangement of the kineties, especially in the oral region, and by superposition of accessory organelles and fibers upon this system of kineties. In the most extensively studied ciliate, Paramecium, this complexity is evident. At least two other ectoplasmic fibrillar systems besides the primary one have been described and the detailed relationships

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of these are disputed (see Taylor, 1941, for review of fibrillar systems in Paramecium and other ciliates). In the oral region the arrangement of fibers and cilia becomes highly complex (Lund, 1933).

Most of the structures described by others using classical methods have been observed in the present study and their relationships have been clarified. However, attention has been directed mainly to the structure and organization of the primary fibrillar apparatus or kineties, with their component cilia, kinetosomes and kinetodesmas. The last are never found to be single "fibers" in the classical sense. As reported briefly elsewhere (Pitelka and Metz, 1952) a kinetodesma is seen to be a bundle of tapering fibrils, each arising independently from a kinetosome and extending for a modest distance along the bundle.

## Materials and Methods

### *Living material and culture conditions*

The following four species of Paramecium were examined in this study: *P. aurelia* (Sonneborn's stock 47, variety 4), *P. multimicronucleatum* (from Kirby's laboratory), *P. caudatum* (from Kirby's laboratory) and *P. calkinsi* (originally from Woodruff's collection). The first three of these were cultured in baked lettuce infusion medium (Sonneborn, 1950) which had previously been inoculated with *Aerobacter aerogenes*. *P. calkinsi* was cultured in identical fashion except that sea water diluted to 2/5 with distilled water was substituted for the distilled water and the $CaCO_3$ was omitted from Sonneborn's formula. With but one or two exceptions only cultures of starved, non-dividing animals were used in the study.

### *Preparation of the material for electron microscopy*

As starting material for the preparation of pellicular fragments rich flourishing cultures of 100 to 300 ml. volume proved to be most satisfactory. When necessary, such cultures were filtered through a loose layer of cotton to clarify the suspension. The culture was then mixed rapidly with sufficient full strength formalin (40% formaldehyde) to give a final formalin concentration of 1 to 2%. The formalin-treated culture was allowed to stand for one to several hours during which time the fixed paramecia settled to the bottom of the vessel. At the end of this period of fixation, the supernatant was removed by aspiration and the concentrated sample of fixed animals was transferred to a 15-ml. centrifuge tube. The procedure employed here has been described in greater detail by Metz (1947) in an unrelated study.

Following fixation the paramecia were washed three times with distilled water (15-ml. washings with mild centrifugation) and finally suspended in 3-5 ml. of distilled water. To prepare the fragments of pellicle this final distilled water suspension was transferred to the receiving cup of a 9 Kc magnetostriction oscillator (Model S–102A, Raytheon Corp.) and vibrated for 30 seconds to several minutes. The duration of this treatment was governed by the degree of dissection desired and the fragility of the individual sample. Some variation was observed in the length of treatment required to break up the animals in different samples. This probably resulted from variations in the fixation procedure. To control these fac-


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tors samples of the suspensions were removed from the receiving cup at 30-second to one-minute intervals and examined with the phase microscope. When the desired degree of disruption had been achieved, the treatment was terminated.

One- to 3-minute treatment usually resulted in thorough disruption of the paramecia. The cilia were stripped from the animals, the pellicles were broken into small fragments and the various formed elements of the endoplasm were released into suspension. Macronuclei, mitochondria and trichocysts in various stages of explosion were frequently observed. Since the present study concerned only the pellicular fragments and attached fibers, the other interesting structures were usually treated as undesirable contaminants and were separated from the pellicular fragments by differential centrifugation insofar as possible. At each step in the procedure the material was examined with the phase microscope. This was found to be necessary to prepare concentrated and relatively pure suspensions of fragments. Small drops of such suspensions were air-dried to collodion-coated electron microscope grids, shadow cast with palladium (*P. calkinsi, P. multimicronucleatum*) or chromium (*P. aurelia, P. caudatum*) and finally examined with the electron microscope (R.C.A. Universal).

*Limitations*

It is apparent that this method of preparation has certain inherent hazards and limitations. The first of these is the usual danger of introducing artifacts by fixation and drying. To minimize this possible source of error, the material was examined routinely with phase optics in the course of preparation and these observations overlapped those with the electron microscope. As a further precaution the fixation procedure was varied by using osmium tetroxide (osmic acid) vapor instead of formalin in a few instances. Within these limits no radical variation was observed in the preparations.

Sonic dissection as used here imposes further limitations on the study. Large numbers of animals were broken up together and fragments were selected for study on a chance basis. No systematic study of a single animal was attempted. To attempt to reconstruct a single organism by direct comparison of such fragments is hazardous and especially so since specific points of orientation are usually lacking in the fragments.

Finally, failure to find a particular structure or structures in these preparations cannot be considered immediate and clear proof of their non-existence in the intact organism, since such structures may have been removed or destroyed by the sonic treatment. The extraordinary feature of the preparations is that so many structures do remain intact.

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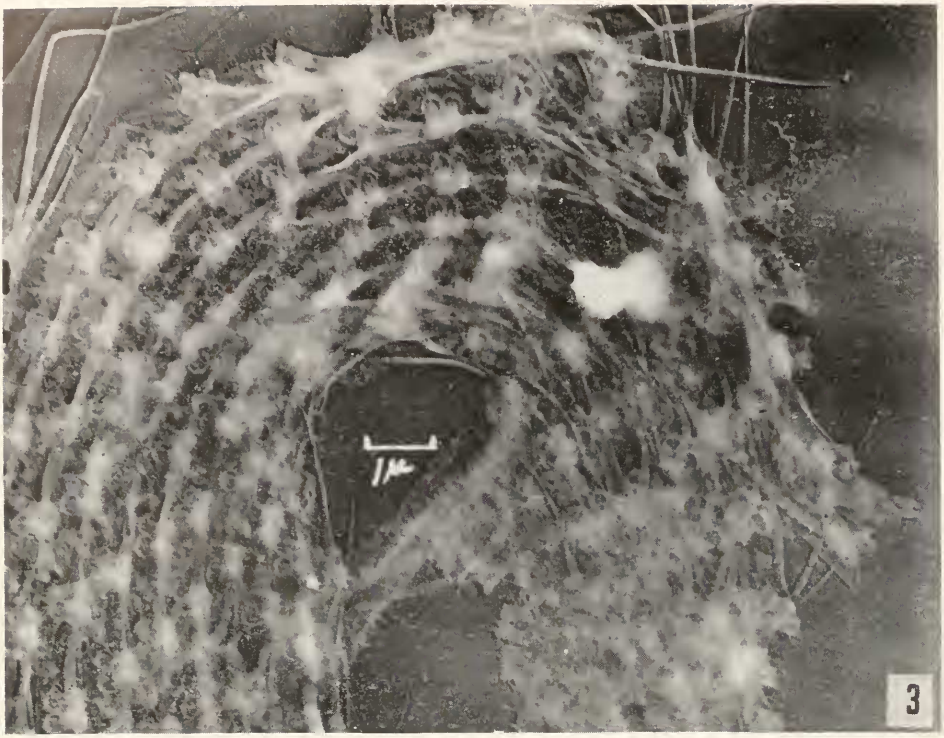
FIGURE 1. *Paramecium aurelia.* A fragment of the pellicle viewed from outside, showing the polygon lattice in the form of hexagons and the subpellicular bundles of kinetodesmal fibrils. Approximately 21,600 ×.

FIGURE 2. *Paramecium aurelia.* A fragment of the pellicle viewed from inside the animal. Remnants of trichocysts are attached to the polygon cross bars (lower left). Single and double (right center) ciliary rings and accessory rings (upper left) appear in the centers of the polygons. Some of the ciliary rings are clearly plugged with cross sectional segments of cilia. Ciliary basal stumps and kinetosomes have disintegrated but the kinetodesmal fibrils remain intact. Approximately 24,000 ×.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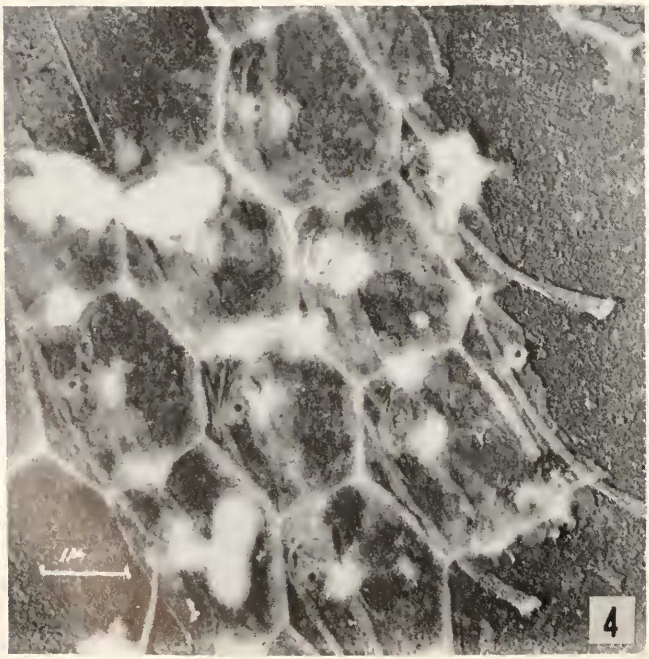
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The reported dimensions of structures and magnification scales must be considered as approximations, since the electron microscopes were not calibrated at regular intervals during the study.

## Results

This descriptive account of the fibrillar systems in Paramecium is based largely on *P. aurelia* and *P. multimicronucleatum*. These two forms have been examined in greatest detail and the study is equally complete for both with the exception that no figures of the oral region of *P. multimicronucleatum* have yet been obtained. Since the two species were not found to differ, the account will apply to both forms.

Figures 1–4 show the type of specimen obtained when the material is properly treated with sonics. The first noticeable feature of the preparations is that the pellicular fragments are nearly free of cytoplasmic material. This might be expected since the pellicles of ciliates may be extracellular membranes (Beams and King, 1941). The really striking feature of the preparations is the collection of fibers that regularly remains attached to these pellicular fragments in spite of the sonic treatment. Indeed, with near minimal treatment, the cilia also remain attached (Figs. 6 and 7). Upon study of Figures 1, 2 and 4 it will be noted that the various structures in the specimens fall into two groups: A) the pellicle and an associated fiber-like lattice and B) a subpellicular fiber system. These are most conveniently considered separately.

### A. The pellicle and pellicular lattice

As seen in Figures 2 and 4 the pellicle proper is a thin membrane. No ultrastructure is evident in this membrane at the magnifications employed. Intimately associated with the membrane is a fiber-like polygonal lattice. The polygons of this lattice usually assume the form of hexagons or quadrilaterals. In some preparations hexagons, quadrilaterals and intermediate forms are all present. With phase optics the polygon lattice appears as a system of ridges. The centers of the polygons are depressed. This fiber-like lattice is certainly the outer fibrillar system figured by numerous authors. The lattice appears as a continuous structure in all electron photomicrographs. No subfibrils or discontinuities are evident. Sedar (1952), however, reports that the material making up the lattice is compound in structure. The only modification in this structural uniformity is found on the cross bars of the lattice. At or near the middle of the cross bars, varying amounts of material remain attached (Figs. 1, 2, 4). In this region the lattice does not dissect clean. This position is described as the trichocyst attachment

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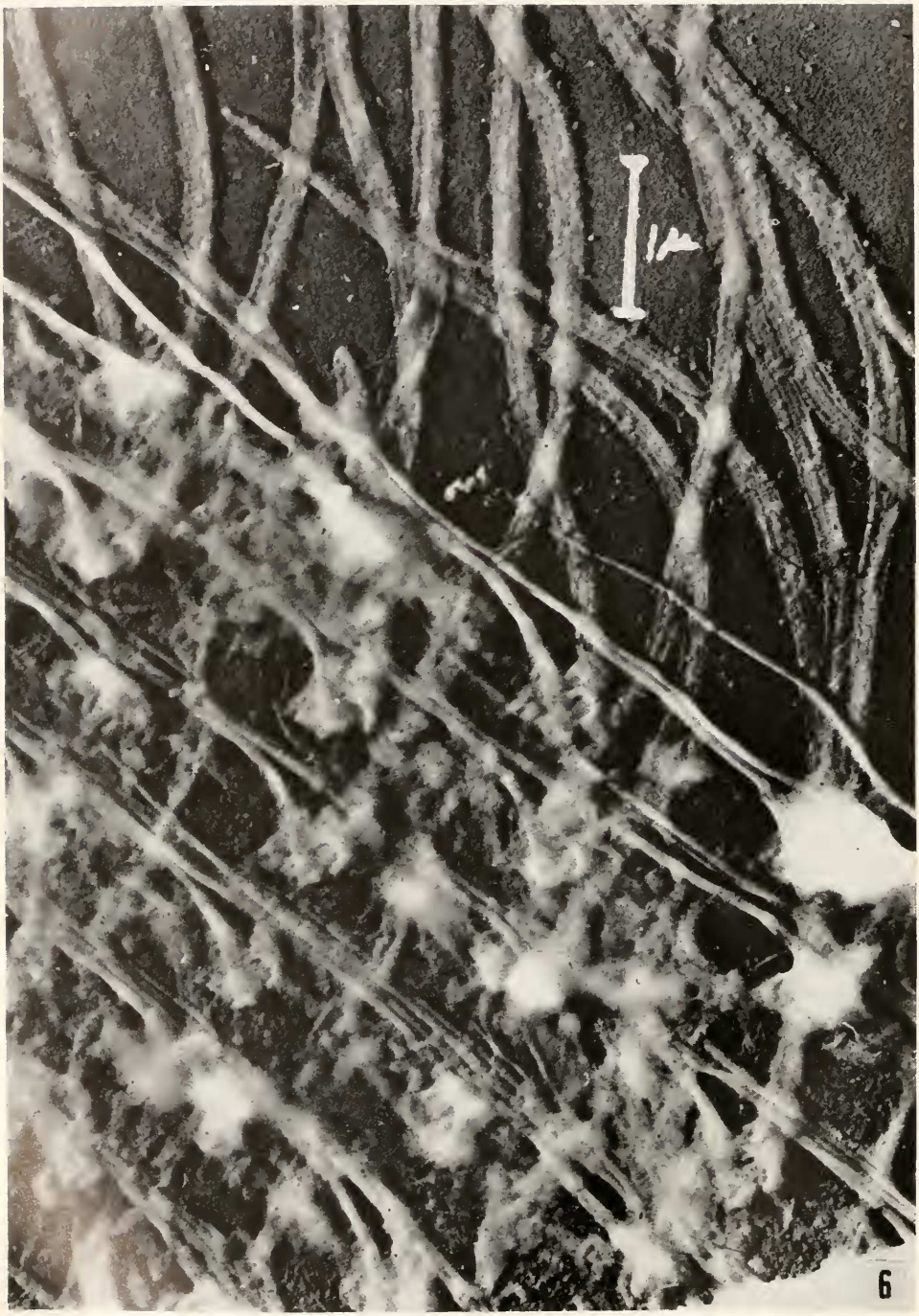
Figure 3. *Paramecium aurelia*. A circumoral fragment viewed from inside the animal. The ciliary rings and kinetosomes are paired. A single kinetodesmal fibril arises from each kinetosome pair. Fibrils run in an anterior direction. Those on the left side curve to the right in the preoral region. Approximately 9800 ×.

Figure 4. *Paramecium multimicronucleatum*. Fragment viewed from outside. Cones with pitted centers occur in the pellicle. These probably are the accessory rings (Fig. 2) seen from the outside. Several kinetodesmal fibrils are not anchored to kinetosomes, yet they remain attached to the kinetodesmal bundle. Approximately 12,000 ×.

Figure 5. *Paramecium calkinsi*. An individual kinetodesmal fibril showing spiral organization. Approximately 48,000 ×.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point by most earlier workers (*i.e.*, Lund, 1933; Sedar, 1952), and is so regarded here. However, it should be noted that in no case was an intact, undischarged trichocyst found attached to a pellicular fragment. In Figure 2 a rather substantial amount of material is attached to one cross bar of the lower left polygon. This may be part of an undischarged trichocyst. The suggestion of a fine fibrillar organization in the structure is of some interest.

The physical relationship between the outer lattice and the pellicle is disputed. Some investigators have held that the lattice is actually a thickened part of the membrane itself, whereas others (most recently Sedar, 1952) maintain that the membrane and lattice are separate structures. Unfortunately, this study does not clarify the issue materially. However, the lattice and the membrane appear to behave as a unit. No specimens were found in which the lattice and membrane were clearly separated. Furthermore, when the pellicle is torn the line of rupture frequently cuts across both membrane and lattice. Therefore, if these are separate structures rather than a continuous one, they must be cemented together rather firmly.

The cilium is generally stated to pass through the depressed center of the pellicular polygon. This view is in agreement with the present findings. Viewed from the inside of the pellicle (Figs. 6, 7) the cilium is clearly seen to pass through a ring-shaped structure. This is believed to be a thickening in the pellicular membrane. Occasionally (Fig. 2) two such rings occur in a single polygon. This situation appears to be constant in fragments from the circumoral area and agrees with the observations of J. von Gelei (1925, 1934a) that two cilia per polygon is a characteristic feature of this region in *P. nephridiatum* and *P. caudatum*. However, Lund (1933) finds only one cilium per polygon in the circumoral region of *P. multimicronucleatum*. It should be recalled that the oral region of *P. multimicronucleatum* was not examined in the present study.

In many preparations a second, smaller ring-shaped structure is found near the ciliary ring. This is seen most readily on an inside view of the pellicle (Fig. 2, upper left). The smaller ring, when present, is constant in position and occurs regularly in many if not all of the polygons in the specimen. The interpretation of outside views in terms of the ciliary ring and the accessory ring is more difficult. In Figure 4 interesting cones with pitted centers are evident. These could be either the external openings of the ciliary rings or the external parts of the secondary rings. The latter possibility is accepted because 1) these cones are very eccentric in position, 2) they do not appear to be associated with the kinetodesmal fibrils to be discussed in the next section, and 3) the opaque structures near the centers of the polygons correspond more nearly to the ciliary rings. These opaque structures are more central in position and larger than the cones; they are associated with kinetodesmal fibrils and finally they occasionally have a suggestion of a larger ring structure. The current investigation gives no hint of a possible function for these secondary rings.

One other structure associated with the pellicle is evident in some preparations. This is a strand or fiber extending across the polygon from one trichocyst attach-

FIGURE 6. *Paramecium aurelia*. Inside view of fragment. Cilia are seen to pass through the ciliary rings in the pellicle and end at the kinetosomes. Kinetodesmal fibrils pass from the kinetosomes and join the kinetodesmal bundles. Approximately 21,000×.


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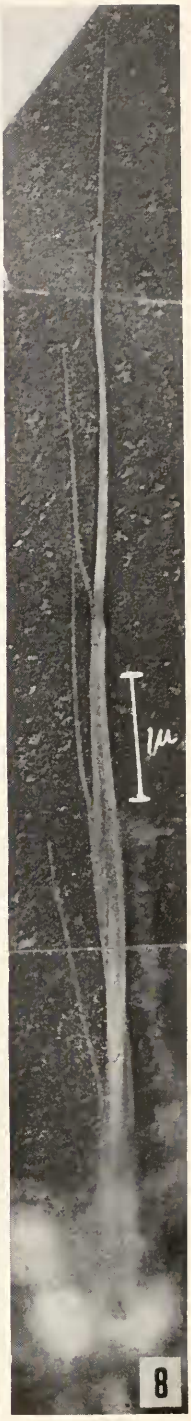
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ment point to the ciliary ring and on to the next trichocyst attachment point. Such structures are seen in Figures 1 and 2. The nature of these is not clear. They appear to be fibers of the same sort and continuous with the lattice fibers in Figure 2. However, they are not present in all preparations, particularly those subjected to prolonged sonic treatment, and their form is somewhat variable (Fig. 1). If this material should take a silver stain, it could well have been confused with the primary fibrillar system to be discussed next.

## B. The primary fibrillar or kinety system

Although the pellicle and the outer lattice are not without interest, the primary system is the striking feature of this study. The essentials of this are clearly illustrated in Figure 6 and consist of the three following parts: 1) the cilium which passes from the exterior through the ciliary ring in the pellicle and extends a short distance into the interior, 2) a bulb or knob at the internal terminus of the cilium, and 3) a long tapering fibril that arises from this knob and joins others of the same sort to form a bundle. This bundle runs parallel to both the surface of the pellicle and the rows of cilia.

### *The cilium*

The cilium itself requires little comment. Its structure, as observed here, agrees with the descriptions of other workers. Thus, the free shaft of the cilium is seen to contain a number of fine longitudinal fibrils and from the studies of Metz and Pitelka (Metz, 1953) it is known to possess a limiting membrane. Unfortunately no clear picture of the proximal origin of the intraciliary fibrils has been obtained in this study although there are indications (Fig. 7) that these fibrils extend to the basal knob. In Figure 2 some of the ciliary rings in the pellicle appear to contain structures that correspond to cross sectional views of the cilium with the contained fibrils. It should be noted that the cilium is never pulled out of the pellicle. When it is broken off, the break occurs at the pellicle. In some cases (Fig. 2) the cilium is broken on both sides of the pellicle. Nevertheless, the ciliary ring remains plugged with a cross sectional segment of cilium.

### *The kinetosome*

In the opinion of the writers, the bulb or knob at the internal terminus of the cilium is the ciliary basal body or kinetosome. This structure has a diameter (*P. multimicronucleatum*) of approximately 0.27$\mu$ (the kinetosome is assumed to have suffered no appreciable flattening since it casts a respectable shadow). This value is about 2.5 times that obtained for the cilium base and the fibril base (0.1 and 0.12$\mu$ in diameter, respectively; calculated on the assumption that these structures are flattened cylinders in the photomicrographs). Comparable values were obtained in *P. aurelia*. When two ciliary rings occur in one polygon, two corre-

FIGURE 7. *Paramecium aurelia*. A segment of the two middle kineties of Figure 6. Approximately 60,800 ×.

FIGURE 8. *P. multimicronucleatum*. A single kinetodesmal bundle extending beyond the torn pellicle. Terminal portions of five component fibrils are shown. These fibrils are seen to overlap in shingle-like fashion. Approximately 15,400 ×.

sponding kinetosomes appear. This is most evident in the oral region (Fig. 3).

In some figures (*i.e.*, 6, 7) the kinetosome seems to be grooved in the direction of the cilium. Otherwise there is no indication of internal structure or multiple organization. No accessory kinetosomes to correspond to Nebenkörner (Klein, 1928; von Gelei, 1932) occur in any of the figures.

*The kinetodesma*

Studies with the light microscope show that the kinetosomes are connected by a longitudinal fiber, the kinetodesma or neuromotor fiber. The electron microscope resolves this into a bundle of fibrils (especially clear in Figs. 6 and 7). Each of the component fibrils in the kinetodesmal bundle arises independently from a kinetosome, passes to one side and joins the main bundle. The kinetodesmal fibrils are not of indefinite length and constant diameter, but taper gradually to end in the main bundle. Thus, the main kinetodesmal bundle consists of a number of fibrils that overlap in shingle-like fashion along the bundle. This is most clearly seen in Figure 8. Here a bundle extends beyond the torn edge of the pellicle. The terminal portions of five component fibrils are clearly shown in this figure. The period between the ends of adjacent fibrils averages approximately 2.3$\mu$. This value compares favorably with the interkinetosome interval (2.0 $\mu$) calculated from another specimen. Evidently then, the kinetodesma of the light microscopist actually consists of a number of fibrils. Each of these arises independently from a kinetosome, joins the bundle and gradually tapers to a fine point. Apparently the kinetodesmal fibrils are of constant length, at least in a limited segment of the bundle. When two ciliary rings with the corresponding kinetosomes occur in a single polygon, both kinetosomes are joined to a single kinetodesmal fibril. This is particularly evident in the oral area.

To judge from the position of the trichocyst attachment points in the outer lattice and the arrangement of the polygons in the pellicular fragments, the kinetodesmal bundles must assume an approximately longitudinal course, with respect to the axis of the animal, except in the oral region. This agrees with the findings of earlier workers (*i.e.*, Lieberman, 1929). Not only do the bundles run longitudinally but the component fibrils are definitely polarized with respect to the axis of the animal. All of the fibrils join the bundle and pass along it in one direction. This is clearly seen in Figures 2, 4 and 6 and is true of every specimen examined that did not clearly represent part of the oral region. In the absence of any anterior or posterior reference point in the fragments it is not immediately clear whether the fibrils run from a posterior to a more anterior region or the reverse. But assuming that the "rule of desmodexy" (Lwoff, 1950) applies in Paramecium, it can be stated with some degree of confidence that the fibrils proceed in an anterior direction from the kinetosomes. According to the rule of desmodexy the kinetosomes lie to the right of the corresponding kinetodesma as the ciliate is viewed in normal orientation. Inspection of the figures shows that the kinetodesmal bundles lie to one side of the kinetosomes and that the individual fibrils curve laterally from their kinetosomal origins to join the main bundle. This condition is probably exaggerated somewhat by the action of surface tension when the preparation is dried. In this connection it should be noted that the kinetodesma appears as a line directly connecting adjacent kinetosomes in most light microscope studies.

In any event, the kinetodesma would necessarily have to deviate sufficiently from a straight course to pass around the trichocyst. Most of the figures presented here are so oriented that the kinetosomes lie to the right of the main kinetodesmal bundle as the fragment is viewed from its outer surface in accordance with desmodexy. In some of the figures (2, 3, 6 and 7) the pellicle is viewed from inside the animal. In these the kinetosomes will appear to the left of the kinetodesmal bundle. When oriented in this way it is apparent that the fibrils pass upward. Thus, if desmodexy applies in Paramecium, it is evident that the fibrils pass in an anterior direction from the kinetosomes.

The arrangement of the circumoral kinetodesmas adds support to this view. At least in the *aurelia-caudatum-multimicronucleatum* group of paramecia the kinetodesmas on the left ventral side of the animal (as viewed from the dorsal surface) pass forward, curve around the anterior margin of the oral opening and finally terminate in the preoral suture (Lieberman, 1929). The kinetodesmas on the right ventral side remain more nearly parallel to the suture. Therefore, the system in the oral region is asymmetrical and an isolated oral fragment can be oriented with respect to its position in the animals. When this is done (Fig. 3) all the kinetodesmal fibrils, on both the left and the right sides of the oral opening, are seen to pass in an anterior direction from their kinetosomes.

*Structure of the individual kinetodesmal fibrils*

No special effort was made in this study to investigate the structure of the individual kinetodesmal fibrils. Nevertheless, certain interesting features are evident in the preparations. These tapering fibrils show a periodic structure when shadowed longitudinally. In the best figures (*P. calkinsi*, Fig. 5) this takes the form of a spiral with a calculated period of approximately 400 Å. The spiral organization extends from the base to the tip of the fibril and its period appears to remain constant throughout the length of the fibril in spite of the fact that the fibril tapers to a point from a base diameter of approximately 0.12 $\mu$ (*P. multimicronucleatum*). It should be noted that the electron photomicrographs reveal this structure as a spiral ridge in the surface of the fibril. This ridge resembles the threads of a screw and casts the shadows which resolve the spiral in the photomicrographs. In order to preserve definition Figure 5 was not "reversed." Therefore, shadows appear light against a dark background in this figure.

The fibril never frays or breaks down in the preparations. It usually remains intact throughout its length in *P. aurelia*, *P. caudatum*, *P. multimicronucleatum*, but breaks off rather frequently in *P. calkinsi*. The fibrils themselves appear to be the most resistant parts of the system. They sometimes break free at their bases from their kinetosomes (Fig. 4) or the kinetosomes and the proximal stumps of the cilia may disintegrate (Fig. 2), but the fibrils remain intact and retain their characteristic organization. A thorough study of these extraordinary structures should prove very interesting. Perhaps more details could be resolved by partial digestion with enzymes or by examining sections at high magnification.

It is not clear how the overlapping fibrils are held together to form the kinetodesmal bundles. In some specimens the individual fibrils appear to be tightly bound together (Figs. 1, 3), but in others the fibrils are separated to a greater or less degree (Figs. 2, 6, 7, 8). Occasionally they even appear as completely iso-

lated structures. Presumably the tightly bound condition is the natural one. The binding agency must involve strong forces, for in many of the preparations (see Fig. 4) a number of fibrils are free from their kinetosomal anchors but nevertheless remain bound to the bundle in normal orientation. No evidence for a sheath about the bundle or any appreciable cementing material between individual fibrils has been found to account for this.

*Commissural connectives*

Some previous investigators have described cross connecting or commissural fibers extending laterally to adjacent kinetodesma. Lund (1933) figures these as connecting kinetosomes and states that they occur occasionally in the general body fibrillar system and that they form a complex lattice in the oral region. In the present study no evidence for such commissural connectives was found outside the oral area. Here, however, individual kinetodesmal fibrils sometimes pass at right angles to one another (Fig. 3). These may represent the commissural connectives of Lund.

## Discussion

Studies on protozoan fibrillar systems are pertinent to several fields of interest. They contribute to the knowledge of protozoan morphology; they apply to the physiological problems of conduction, coordination and contraction; and they concern problems of morphogenesis. These aspects of the present investigation are considered below.

*Morphological considerations*

The main features of the pellicular lattice and kinetodesmal system of Paramecium, as seen in this study, agree with the findings of most previous workers. Indeed they confirm the views of J. von Gelei (1925), Lund (1933) and Worley (1933) in a most striking manner. The outer lattice is quite evidently distinct and anatomically separate from the primary or inner system. The only possible exception to this could be a connection between the trichocyst attachment point and the cilium ring in the pellicle. No evidence for any direct connection between the trichocysts and the kinetodesmal system was found. The study does not support Klein's (1926) concept of an indirect connection between the lattice and kinetodesmal system.

Certain other fibrillar structures have been described in Paramecium. Rees (1922) reported a system in which fibers arise from the kinetosomes, pass into the endoplasm and then proceed more or less directly to a neuromotorium located in the vicinity of the cytopharynx. No suggestion of the fibers of Rees was found in the electron photomicrographs. G. von Gelei (1937) has described still another fiber system. This constitutes a lattice at the level of the kinetosomes which ramifies throughout the animal at this level. No evidence for this system appeared in the current investigation. Although this study does not confirm the existence of these structures, it cannot be considered as critical evidence for their absence in view of the method of preparation of the material (see section on methods).

A number of investigators have described the kinetosome region of Paramecium as a compound structure (Klein, 1928; J. von Gelei, 1932; Sedar, 1952) containing

two or more distinct granules. One of these is usually considered to be a true ciliary basal body; the remainder as accessory bodies or Nebenkörner. In three of the four species examined with the electron microscope the kinetosomes appear to be single structures. In the exceptional form, *P. calkinsi,* two bodies which may be kinetosomes are frequently associated with each pellicular polygon. The study of this form is not sufficiently complete to determine whether this condition is general over the body surface or whether it applies only to the oral region as in *P. aurelia.* Likewise the detailed relationship of these bodies to the kinetodesmal fibrils in *P. calkinsi* has not yet been worked out.

Possibly compound kinetosomes may yet be found in *P. aurelia, P. multimicronucleatum* and *P. caudatum* after sonic treatment if the fixation procedure is varied. But whether or not such accessory structures are found, there is no question that at least one such structure joins the base of the cilium to the kinetodesmal fibril and that bundles of these tapering fibrils overlap to form the kinetodesma of the light microscopist.

Concerning this relationship of kinetosome to the cilium and kinetodesma, Worley's (1933) Figure 2 is of interest. This figure, a photomicrograph of a fresh preparation, clearly shows a series of bodies from which short fibers arise. These fibers appear to overlap. Worley makes no comment concerning this overlapping condition although he does state that direct connecting fibers (kinetodesma) are visible in the figure. However, it appears likely that these fibers are cilia which all lie in one direction.

*Physiology*

Nearly every student of Paramecium fibrillar systems has attributed specific functions to the various fibrillar structures. These functions include physical support, contraction, specific metabolic activity and various aspects of ciliary coordination. Unfortunately, few investigators have made any experimental attempt to support their theses, but rather follow the procedure of Lund (1933) who, on the basis of morphology alone, concludes (p. 58) that a correlating function "is almost to be accepted without choice" in the case of the kinetodesma. The most serious attempt to investigate the function of fibrillar systems in Paramecium by experimental means appears to be the study of Worley (1934).

Employing microdissection methods, this investigator made short transverse cuts through the ectoplasm of Paramecium and noted that the metachronal waves of ciliary action did not proceed beyond the cut. Metachronism was not disturbed elsewhere. Evidently, control of metachronism resides in the ectoplasm and the control follows longitudinal paths. In Worley's experiments ciliary reversal occurred simultaneously over the entire body surface and on both sides of ectoplasmic cuts. This suggests that the kinetodesmal fibrils do not control ciliary reversal. Although this evidence is neither extensive nor overwhelming, it does support the view that the kinetodesmal system has a role in ciliary coordination. For more extensive treatment of this aspect of the subject the reader should consult Parker (1929), Taylor (1941), Prosser *et al.* (1950) and Wichterman (1953).

The present demonstration that the kinetodesmas are not single fibers but consist of bundles of relatively short overlapping fibrils, each connected independently to a kinetosome, introduces a complication in any coordination theory of kineto-

desmal action. Coordination would have to pass laterally from one fibril to another— presumably to the fibril of the next adjacent cilium. This would imply a high degree of orientation of the fibrils with respect to each other and also that the relationship has the properties of a lateral synapse. In this connection it is of interest that *Tetrahymena* contains a specific acetylcholine esterase (Seaman and Houlihan, 1951) and that this enzyme appears to be confined to the ectoplasm of the ciliate (Seaman, 1951).

Many ciliates are highly contractile organisms. Although Paramecium is not notable in this regard, some investigators (Brown, 1930) have ascribed a contractile function to certain of the fibrillar elements. If Paramecium showed clear-cut contractile properties, it would be tempting to ascribe such action to the spiral organization of the kinetodesmal fibrils.

*Morphogenesis*

In ciliates the kinetosomes appear to be self-reproducing, genetically continuous entities; in certain forms they may act as pluripotent organization centers and finally they may be the seat of morphogenetic dominance and polarity (Lwoff, 1950; Weisz, 1951).

In Paramecium the demonstrated behavior and properties of the kinetosomes are somewhat less spectacular. During fission or as a preliminary to fission all body kinetosomes divide one or more times, the daughter granules separate along the longitudinal axis of the animal and finally form new cilia (von Gelei, 1934b; Downing, 1951). The kinetosomes of the oral region may be responsible for the specialized oral morphology and ciliature, since the new oral region is formed by a budding process from the original at fission (Hertwig, 1889; von Gelei, 1934b). The morphogenetic agents in the bud could be kinetosomes although evidence for this view is lacking. The origin of trichocysts is obscure (Wichterman, 1953); von Gelei (1934b) believes that new trichocyst granules arise from the neuronemes (kinetodesmas), a view which agrees roughly with that of Lwoff (1950) for apostomatous ciliates.

Although the present study was not designed specifically to clarify morphogenetic problems, it does re-define certain of these at a finer morphological level. This applies particularly to the problems of duplication and polarity of kineties. An individual kinety is seen to be a compound structure composed of definite units. Each unit is composed of a cilium, a kinetosome and a short, tapering kinetodesmal fibril. How, then, do these units duplicate and orient to form the compound structure of the kinety? According to current views the kinetosome is the primary duplicating organelle. By unknown mechanisms it can reproduce itself and it can produce other organelles. In keeping with this concept an individual kinetosome of Paramecium should be able to reproduce itself and produce a cilium with its complex internal structure. Ninety degrees from the site of cilium origin it should produce a kinetodesmal fibril with its taper and spiral symmetry. In the course of this study a single forked kinetodesmal fibril with two tapering ends was encountered. This unusual fibril could have arisen as a "developmental anomaly" from a kinetosome, but it is also possible that duplication is regularly initiated at the tip of the fibril and proceeds proximally to include the kinetosome.

In all of the preparations examined the kinotodesmal fibrils are polarized. They all taper in one direction and form tight bundles. If the kinetodesmal fibrils develop from the kinetosomes, "contact guidance forces" (Weiss, 1945) of some sort might cause a developing fibril to proceed along a pre-existing bundle, but such a concept does not so readily explain the exclusive anterior orientation in this polarity.

Refinement of the methods used here and their application to a variety of forms in stages of division and regeneration, in conjunction with sectioning techniques, may be expected to provide some insight into these problems.

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## Summary

1. Various structures in Paramecium are readily obtained for electron microscope examination by formalin fixation followed by sonic dissection. These structures include macronuclei, mitochondria, trichocysts in various stages of explosion and fragments of the pellicle. This study concerns such fragments.

2. The electron microscope reveals two fiber systems associated with the pellicular fragments. One of these is intimately associated with the pellicular membrane and corresponds to the outer fibrillar lattice system of earlier investigators. The second system is subpellicular and corresponds to the kinety, neuroneme, or inner fiber system.

3. The pellicle proper consists of a thin membrane with no obvious fine structure. The outer lattice system is a continuous network. It corresponds in position to the system of polygonal ridges in the pellicle. A thickening in the cross bars of the polygonal lattice represents the trichocyst attachment point. A ring-shaped structure is found in the center of each polygon. The cilium passes through this ring. A short distance from this ciliary ring a second, smaller ring-shaped thickening is frequently found.

4. The kineties are compound structures composed of discrete units. Each unit consists of three parts: a) the cilium which passes through the ciliary ring of the pellicle and terminates internally at b), the ciliary basal body or kinetosome. Each kinetosome gives origin to c), a tapering fibril which parallels the surface of the animal. These units are associated by their tapering fibrils. The fibrils from a longitudinal row of kinetosomes overlap in shingle-like fashion to form a tight bundle. This bundle is the kinetodesma of the light microscopist. The fibrils of the kinetodesma all taper toward the anterior end of the animal.

5. No obvious connection exists between the outer lattice system and the kinety system.

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